

ABSTRACT OF THE DISCLOSURE:

The invention is a procedure for measuring the binding of an entity (ligand) to a
4 surface by using a hapten-conjugated version of the ligand (hapten-ligand). An excess of
the hapten-ligand is presented to the binding surface and excess (unbound) hapten-ligand
is washed off. Bound hapten-ligand is then solubilized (removed) and applied to a
membrane support or separated by electrophoresis and applied to a membrane support.
8 Known amounts of hapten-ligand are similarly applied to the membrane, to provide for
hapten-ligand standards. The membrane-bound hapten-ligand is detected by application
of an enzyme-conjugated antibody to the hapten; or by application of an antibody to the
hapten followed by application of an enzyme-conjugated antibody to the anti-hapten
12 antibody. The resultant membrane-associated enzyme is detected and quantitated by the
application of a color or light-producing substrate which reacts with the enzyme. Results
obtained from the standards are used to calculate the amount of hapten-ligand in the
membrane areas corresponding to the unknowns. Thus, the amount of hapten-ligand
16 originally bound to the surface can be determined. A combination of the use of anti-
hapten antibodies along with membrane-blotting technologies to assess hapten-ligand
binding to surfaces is not found in the scientific or patent literature, particularly in
regards to assessing protein binding to cell surfaces.